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The present paper comprises a summary of earlier investigations, which have been published in the following:

A. B. Sjöström: The activity of prothrombin and proconvertin in plasma in diseases of the biliary tract. A clinical study. Acta chir. Scandinav. Suppl. 223.

B. S. Falkmer and B. Sjöström: Histological changes in the liver in diseases of the biliary tract. Gastroenterologia 91: 221, 1959.

C. K-E. Arosenius and B. Sjöström: The activity of prothrombin and proconvertin in plasma in acute cholecystitis. In press.

In the following these articles will be referred to using the letters given above.

Introduction

One of the functions of the liver is to synthesise the coagulation factors prothrombin and proconvertin which form an integral part of the prothrombin complex. This function can be disturbed by causes, which may schematically be divided into extrahepatic and intrahepatic, although they are often combined and difficult to distinguish clearly. A purely extrahepatic cause of lowered prothrombin-proconvertin synthesis, however, may be an insufficient absorption of vitamin K due to biliary obstruction. Such a condition can produce cholaemic bleeding, the clinical pattern of which has been known for a long time (Petrén, 1925). As examples of reduction in prothrombin and proconvertin values with a purely intrahepatic pathogenesis may be cited hepatitis and cirrhosis (Owren, 1949). A similar functional disturbance may also be found in certain cases of cholecystitis (Hultén, 1954), the morphological equivalent of which may possibly be the demonstrable changes in the liver parenchyma in cholecystitis (Graham, 1953; Ågerup, 1950; Hultén, 1954 etc.).

In the present paper, an account will be given of disturbances in the prothrombin-proconvertin content of plasma and histological changes in the liver parenchyma in cholelithiasis and cholecystitis on the basis of the results obtained in A, B and C. The role of inflammation of the gallbladder as an aetiological factor will also be analysed.

Methods

The determinations of the combined activity in plasma of prothrombin and proconvertin (p.p.) have been performed by methods (A) similar to those for measuring the isolated activity of these coagulation factors (pt. and pc. respectively), and which to all intents and purposes correspond with those described by Owren and Aas (Owren, 1949; Owren and Aas, 1951; Aas, 1952). In my experience the error of the methods for determining p.p., pt., and pc. has been about 1.5 % of the observed value. The methods may therefore be considered reliable.

A tolerance test on latent disturbances in p.p., pt., and pc. (A) has also been used. In this test a 0.75 % solution of the sodium salt of warfarin was used to induce hypoprothrombinaemia and hypoproconvertinaemia. The mean p.p., pt., or pc. on two consecutive days was taken as initial value. The warfarin-sodium solution, at a dose of 0.125 mg per kg body weight, was injected intravenously after sampling on the second day, and the values recorded on each of the following four days. A horizontal base-line is drawn from the initial value, and those of the 1st, 2nd, 3rd, and 4th days' values that lie below this level are subtracted: the sum of the resulting figures represents the surface below the base-line and limited by this and the values for the several days. The surfaces are designated s.p.p., s.pt., or s.pc., according to the activity of the coagulation factor or factors concerned, and expressed in relative units (rel.u.).

Pathologically low values of p.p., pt., and pc. were considered as being present with deviations of 2 σ or more from the mean in a control series. On this basis, values of 70 % and below for all three factors are considered pathological (A). Values between 71 % and 80 % were considered as "possibly pathological" in interpreting the statistical relation between the graded histological changes and the p.p. (B), since about 95 % of the values in the control-group lie above 80 %. It was considered that the tolerance tests were pathological if the values had a deviation of at least 3 σ from the mean of a control series. According to these criteria, s.p.p. of 135 or more rel.u., s.pt. of 180 or more rel.u., and s.pc. of 150 or more rel.u. may be regarded as pathological (A). Values between 90 and 135 rel.u. of s.p.p. were regarded as "possibly pathological" (B), since no value in the control-group exceeded 90 rel.u.

Concerning other laboratory tests carried out please refer to papers A, B and C.

The biopsy material was obtained in the following way. As soon as the abdominal wall was opened, a wedge-shaped excision 2 cms. deep was made in the liver parenchyma in the right lobe as far as possible from the gall-bladder bed (B). The liver biopsy specimens were routinely fixed in 4–10 % formalin. Van Gieson's stain and hematoxylin and eosin were used as routine stains. All sections were examined as unknowns on repeated occasions by the same observer. A semi-quantitative estimation of the histological changes was made in four grades: 0, 1+, 2+, and 3+.

The activity of plasma prothrombin and proconvertin in cholelithiasis and cholecystitis

Without the use of tolerance tests (Hultén, 1954), no reduction in plasma activity has previously been found in investigations of the status of prothrombin in cholelithiasis and cholecystitis with measurement of the whole complex (Mateer, Hartman, Baltz, Fallis, McGraw, and Steele, 1948). The methods for specific measurement of the factors in the prothrombin complex, however, are more sensitive than those used for the whole complex (Björnesjö and Karlström, 1958). Disorders in the synthesis of the prothrombin complex in cholelithiasis and cholecystitis previously unknown, could be revealed by means of these specific methods.

Thus in paper A, it was shown that in certain patients with cholelithiasis and cholecystitis is found a decrease in p.p., pt., and pc., as signs of disordered liver function. The series of patients has been divided up so that the frequency of such lowered activity is seen to be greatest in patients with cholecystitis and jaundice (A: chapter 3, pp. 24–26).

Lowered p.p. values occurred in patient groups I and II, patients with inflammatory biliary disease with jaundice and with inflammatory biliary disease without jaundice to the extent of about 15 %. In patient group IV, patients with non-inflammatory biliary disease without jaundice, the frequency of such values was only 4 %. There was also a group of patients with icterus from biliary obstruction (group III) where the frequency of lowered p.p. values was high. The finding in this group confirms what was known already, but it has no significance in answer to the question of the role of inflammation of the gall-bladder in causing the falls in p.p. pt., and pc. in the other groups of patients.

The means of p.p., pt., and pc. in group I were lower than the corresponding controls with statistical certainty. The disorder, as well as the icterus in this group, was therefore considered to depend on a disturbance of liver function following on inflammation of the gall-bladder. Icterus with this origin has previously been described (Ågerup, 1950; Hultén, 1955). As a support for the idea that the icterus in this case was also of inflammatory origin and not obstructive, it was stated that no statistically significant correlation existed between common

duct stones found at operation and bilirubinaemia in groups I, II and IV. A vitamin K deficiency from biliary stasis could not be considered responsible for the disturbance since no statistically significant correlation existed between common duct stones found at operation and decreased p.p. values. The role of inflammation in the origin of lowered p.p., pt., and pc. was further elucidated by the tolerance test on latent disturbances. Values were obtained (A: chap. 3, pp. 38–40 and chap. 4, pp. 47–48) which with statistical certainty were different from the control series both for inflammatory non-icteric, and for inflammatory icteric patients. Thus a heightened sensitivity to warfarin of about 48 % occurred in group I and of 20 % in group II. Group IV showed only 7 % pathological values and this group's mean of s.p.p. was not significantly higher than the control. There also existed a statistically certain difference between the means of s.p.p., s.pt., and s.pc. in groups I and IV, thus between patients with both cholelithiasis and cholecystitis and those with only cholelithiasis.

However, complete certainty has not been achieved concerning the role of inflammation, before patients with stone-free common bile ducts (viz. "pure cases") have been studied. The report of such a survey of cases follows below and thus comprises only patients, whose common duct was free of stones at operation.

Investigation of prothrombin-proconvertin synthesis in "pure cases" in the series in paper A. Of the disorders measured in pt. and pc., those of pc. are greater. A fall in pc. influences the combined activity of the factors so that measurement of the combined activity is sufficient to reveal a disturbance (A: chap. 3, pp. 26, 31, chap. 4, pp. 47–48). On going through the "pure cases" in this series of patients, only p.p. values have been considered. The values from samples taken on arrival at hospital have been used, where such exist. In other cases, values have been used, measured after sampling shortly before operation. When the values were compared, those from patients with diabetes and pernicious anaemia were removed, as it is known that such illnesses can be associated with disorders in liver function (Owren, 1953; Popper and Schaffner, 1957). The series of patients has also been reduced somewhat owing to the lack of data in some of the case-histories when they were gone through. The "pure cases" comprise 39 group I, 47 group II, and 24 group IV patients, together with 35 of groups I, II and IV, whose prothrombin-proconvertin synthesis was examined with warfarin tolerance tests.

The means of the "pure cases" for p.p. in groups I, II and IV are seen in the table, which shows a statistically significant difference between those in groups I and II and the control series and reciprocally between I and IV. The earlier comparison (A: chap. 3 p. 24) gave a significant difference only between the control series and group I, while the difference between the control material and group II and reciprocally between I and IV were only statistically significant with the tolerance test. The number of pathological p.p. values are 7/39

Table. Mean values of p.p. (%) and s.p.p. (rel.u.) in groups I, II and IV ("pure cases") and the differences between the mean values of the control series (C) and of the patient-series (P).

Group	p.p.		s.p.p.	
	$M \pm \varepsilon_M$	Diff C-P	$M \pm \varepsilon_M$	Diff P-C
I	83.3 ± 2.0	11.8 ± 2.1	113.7 ± 19.4	87.0 ± 19.7
II	88.2 ± 1.8	6.9 ± 2.1	88.9 ± 16.3	62.2 ± 16.7
IV	94.3 ± 1.8	0.8 ± 2.0	47.8	21.1

in group I, 6/47 in group II and 0 in group IV, the corresponding frequencies in groups I and II being about 18 and 13 % respectively. The frequency of pathological values in group I is thus greater than in the earlier series while that in group II is almost unchanged (A: chap. 3, p. 26). The "pure cases" in this part of the patient series, who were examined with the tolerance test, show mean values, lying at the same level as those in the earlier series (A: chap. 3, p. 38) and are significantly higher in groups I and II than the mean of the controls. S.p.p. are 113.7 ± 19.4 rel.u. in group I and 88.9 ± 16.3 in group II. In group IV the mean of 6 s.p.p tests performed was 48 rel.u. and insignificantly different from the control 26.7 ± 3.7 rel.u. (Table). The number of pathological s.p.p. values in group I is 4/10, in group II 6/19 and in group IV 0, thus about 40 % and 31 % respectively in groups I and II. In the earlier series, the corresponding figures were 48 % and 20 % respectively (A: chap. 3, p. 40). The results now obtained make it completely clear that inflammation of the gall-bladder is responsible for the falls in prothrombin and proconvertin synthesis found here and that the conclusions drawn from the earlier series including cases of stone of the common bile duct, were correct. Vitamin K in therapeutic doses brought all the reduced values in p.p. back to normal (A: chap. 3, p. 25), although these disorders should not be considered as signs of vitamin K deficiency. In animal experiments vitamin K_1, in large doses, has been shown capable of restoring the prothrombin content in plasma despite considerable liver damage following the administration of CCl_4 (Jürgens and Studer, 1952).

Investigation of prothrombin-proconvertin synthesis in patients with stone in the common bile duct in the series in paper A. The cases with stone have also been examined. The frequency of pathologically reduced p.p. values is lower in groups I and II than in the "pure cases": 3/20 or about 15 % in group I and 1/12 or about 8 % in group II. Surprisingly enough, the means here are higher than those of the "pure cases". Thus group I patients show a mean p.p. value of 89.0 ± 3.7 % and group II patients one of 98.0 ± 3.7 %.

There are no statistically significant differences here in comparison with the control series, nor for mean p.p. values of 12 cases from these combined non-inflammatory groups III and IV; 92.2 ± 8.0 % with a pathologically lowered value in group III. However, the cases of stone in the common duct from groups I to IV in the tolerance tested part of the series, have a mean value for s.p.p. which is significantly higher than that in the control material, viz. 92.8 ± 15.7 rel.u., and there are 4 pathological s.p.p values in group I but none in groups II, III and IV. The high mean values for p.p. need not therefore express a reactive hyperprothrombinaemia, which has been considered to occur with incomplete biliary obstruction (Witte and Dirnberger, 1953).

Prothrombin-proconvertin synthesis in acute cholecystitis. Patients with cholecystitis in the acute stage have not been combined into a unified group in paper A, whereas the purpose of the investigation described here was to find out the occurrence of disturbances in prothrombin and proconvertin synthesis in biliary disease in a larger over-all series. When the role of inflammation was classified through the previously described investigation and through the investigation described above of the "pure cases" of that series, it is necessary to study the conditions in patients with signs of existing inflammation, such as fever, tenderness and leucocytosis. This has been carried out in the investigation, described in paper C. From the results there it can be seen that p.p. falls in cholecystitis are more usual (frequency about 45 %) than in the earlier series and at times also more pronounced. The p.p. means here lie at about the same level as those in group I of the "pure cases": 79.8 ± 4.9 % compared with 83.3 ± 2.0 %. This agreement in means implies that the "pure cases" in group I consisted almost entirely of acute or subacute cholecystitis patients, in whom the in group I actual icteric form occurs most commonly (Hultén, 1955). That the frequency of pathological values is so much higher in the series of acute cholecystitis than in group I implies that disturbances in the prothrombin-proconvertin synthesis is intimately associated with the course of the illness: all the acute cholecystitis patients (C) have been examined for p.p. on arrival at hospital while in many of the "pure cases" in group I (A) sampling was not performed until shortly before operation. No acute operations have been taken in group I. The majority of patients, who among the acute cholecystitis cases showed normal p.p. values, have been examined with the tolerance test and have shown a disturbed prothrombin-proconvertin synthesis in about 50 %.

Prothrombin-proconvertin synthesis in chronic and subacute cholecystitis. The p.p. and s.p.p. values, which were combined as biopsy material in paper B are derived from samples in connection with operations, which were performed in a symptom-free interval. Thus these values should reflect prothrombin-proconvertin synthesis in chronic and subacute cholecystitis and show (B: p. 11) a frequency of pathological values in groups I and II, icteric and non-icteric

patients with inflammatory biliary disease, which clearly implies that the disorder in prothrombin and proconvertin synthesis can remain during the whole course of the illness. 16 tolerance tests carried out gave a frequency of pathological values and a mean, similar to those in group I in paper A: about 43 % pathological, mean 117 rel.u. The falls in p.p. values are of the same order as in the series in paper A with a mean of 67.2 % and 64.4 % respectively, compared with 65.2 % and 67.7 % (A: chap. 3, p. 26). However, the means of p.p. for all patients in groups I and II in the biopsy series lie higher, 95.0 ± 1.8 % and seem to imply that the synthesis of the actual factors was constantly better in these patients.

Histological changes in the liver in cholelithiasis and cholecystitis

Morphological changes in the liver in cholelithiasis have previously been described (Graham, 1933; Ågerup, 1950; Edlund, 1952; Ekdahl and Stenram, 1958 etc.) but their distinct occurrence has also been denied (Colp, Doubilet, and Gerber, 1935; Keller and Smetana, 1950; Edlund and Zettergren, 1957). However, Edlund and Zettergren point out that "cloudy swelling" is commoner in acute than in chronic cholecystitis. This liver change, however, is not generally accepted (Popper and Schaffner, 1957). Disorders in liver function arise in certain cases of cholecystitis (Batty and Gray, 1943; Mateer et al., 1948; Ågerup, 1950; Hultén, 1954) and attempts have been made to correlate these with the histological changes found. No correlation could be shown between disorders in prothrombin activity and liver changes (Mateer et al., 1948; Popper, Steigman and Szanto, 1949) but some correlation was shown between the results of certain flocculation tests and liver changes (Mateer et al., and Ågerup).

Liver changes in the author's own biopsy material. A series of biopsies has been briefly described in paper A. In this series, it was apparent (A: chap. 3, p. 41) that liver changes such as diffuse liver cell changes, periportal inflammatory reaction, fatty change and "cholecystogenic hepatitis" occurred in every group of patients. Changes were commoner in biopsies from patients with inflammation of the biliary tract, groups I and II, than in those from non-icteric non-inflammatory patient groups, group IV. The division of the pathological changes in the patient groups was thus similar to that of pathological p.p. values, so that it was assumed that a connection existed.

Because of the contradictions in the literature concerning liver changes in cholelithiasis and cholecystitis it was thought necessary to examine a larger series. The changes in such a biopsy material could be graded and then examined on whether a significant statistical difference could be confirmed between

biopsy findings in patients with inflammatory and non-inflammatory cholelithiasis. Similarly the possible occurrence of statistical correlation between histological changes and functional disturbances should be investigated. Such an investigation has been described in paper B.

As in paper A, the patients had been divided up into groups I–IV: Inflammatory biliary disease with jaundice (I), Inflammatory biliary disease without jaundice (II), Non-inflammatory biliary disease with jaundice (III), and Non-inflammatory biliary disease without jaundice (IV). In this biopsy material, the occurrence of the following changes has been shown and graded: portal inflammatory reaction, diffuse liver cell changes, the presence of extra- and/or intracellular pigment, fatty metamorphosis of the liver cells, nuclear vacuolation, focal necrosis, and portal fibrosis (B: pp. 4, 10). Apart from the changes, portal inflammatory reaction, the presence of extra- and/or intracellular pigment, and portal fibrosis, changes appeared most commonly in biopsies from patients with inflammation of the biliary tract. Portal inflammatory reaction was more common in groups I and II than in group IV but appeared also as a common change in group III. Changes appeared as often as in the series described in paper A (A: chap. 3, p. 41). There was a statistically significant difference between diffuse liver cell changes in inflammatory and non-inflammatory groups of patients. No such difference could be shown in paper A. The difference found now implies that inflammation of the gall-bladder is responsible for these liver changes.

Correlation between functional disorders and histological changes. In calculating correlations between the histological changes found and disorders in prothrombin- and proconvertin synthesis, a statistically significant correlation was found to exist between the occurrence of extracellular pigment and lowered prothrombin-proconvertin activity. A probably significant correlation was also found between the occurrence of focal necrosis and lowered prothrombin-proconvertin activity (B: p. 13). This latter correlation is of the greatest interest concerning cholecystitis, where the occurrence of focal necrosis was most common.

Since the correlation with the method used in paper B was only probably significant, it has been further investigated. It is seen to be significant in group I where a statistically significant difference exists ($P < 0.001$) between the means of p.p. in patients, whose biopsies showed focal necrosis (grade $\geq 1+$) and in such patients, whose biopsies lacked focal necrosis (72.2 ± 3.3 % and 106.8 ± 3.2 % respectively). This correlation confirms the fact that disorders in plasma prothrombin and proconvertin activity in disease of the biliary tract with inflammation have a histological equivalent in the liver. Thus focal necrosis may be considered as a sign of parenchymal damage, causing a disorder in liver function. Of the other laboratory tests increased bilirubin values showed correlation with diffuse liver cell changes, with the combination of diffuse liver

cell changes + portal inflammatory reaction, and with the occurrence of extra- and intracellular pigment. Increased alkaline phosphatase values showed a correlation with the same changes except diffuse liver cell changes. An increased E.S.R. showed a probably significant correlation with nuclear vacuolation. None of the laboratory tests showed a correlation with portal inflammatory reaction and fatty metamorphosis.

Concluding remarks

A disorder of prothrombin and proconvertin synthesis has been shown to occur in both acute and chronic cholecystitis without obstruction of the common duct. Fall in activity of these coagulation factors is most common and most pronounced in acute cholecystitis but is also found in chronic and subacute cholecystitis.

This disorder could not be revealed in previous investigations where the activity of the whole prothrombin complex was measured (Mateer et al., 1948). The methods used by the author (Owren, 1949) are more sensitive than those for the measurement of the whole prothrombin complex (Alagille, 1956; Björnesjö and Karlström, 1958). *In order to reveal latent disorders in the synthesis of prothrombin and proconvertin, the author has introduced a warfarin tolerance test* the development of which was based upon the previously used dicoumarol tolerance test (Saracbasi and Inceman, 1950; Hultén, 1954). With this test, it has been shown that after intravenous supply of a certain dose of warfarin sodium, a fall in p.p. values occurred in many gall-bladder patients. These falls are greater and more prolonged than in healthy people and as stated above reveal a latent disturbance in the synthesis of prothrombin-proconvertin in those patients. This test, like simple measurement of prothrombin-proconvertin activity, could thus be a very useful test of liver function.

Histological liver changes occur in cholecystitis, to judge from the author's biopsy material. Such liver changes have also recently been described by other authors (Cole and Elman, 1959). The liver changes are interpreted as a reaction to the spread of the inflammatory process in the gall-bladder. The spread in cholecystitis could occur via the blood- or lymph-stream (Graham, 1933; Voegt and Weyer, 1956). In addition, with obstruction of the common duct, spread via the duct itself is possible (Selberg, 1955). Liver changes have been shown to regress after cholecystectomy (Albot and Gibelin, 1957).

The reaction in the liver, following inflammation of the gall-bladder thus manifests itself partly in functional disorders and partly in histological changes. As evidence for this it may be said that in the author's statistically treated biopsy material there exists a correlation between tests, which imply functional disorder and liver changes. *A significant correlation exists between disorder in plasma prothrombin-proconvertin activity and focal necrosis of the liver.*

The parallelism between the histological changes and the functional disorders imply that even the latter are reversible.

The author considers that the results obtained are in favour of the early removal of an inflamed gall-bladder. However, the trauma of operation can make worse an already existing deficiency in prothrombin-proconvertin synthesis and also make manifest a latent disorder of this synthesis. If the operation is performed in the acute stage, vitamin K should therefore be given prophylactically, to avoid those complications, arising from an increased bleeding tendency.

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